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## Effects of water quality on survival and reproduction of four species of planaria (Turbellaria: Tricladida)\*

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### Summary

In a series of bioassays, four planarian species — *Dugesia dorotocephala* (Woodworth), *Dugesia tigrina* (Girard), *Cura foremanii* (Girard), *Dendrocelopsis vaginatus* (Hyman) — were evaluated against six water quality parameters (hardness, temperature, salinity, pH, dissolved organics, and dissolved oxygen) for effects on species survival and asexual reproduction. No significant ( $P < 0.05$ ) effects were determined on survival and asexual reproduction of three species exposed to various levels of hardness. *Dugesia tigrina* was determined to have a significant increase in asexual reproduction with an increase in hardness. Dissolved oxygen was determined to be a critical parameter affecting both survival and reproduction of the species evaluated. *Dugesia dorotocephala* and *D. tigrina* were determined to be the most adaptable and tolerant of the species evaluated.

**Key words:** Planaria, water quality, survival, reproduction

### Introduction

Planaria (Turbellaria: Tricladida) are primarily free living, marine, freshwater, and terrestrial flatworms (Platyhelminthes) (McConnell, 1967). Selected species have been reported to be effective predators of aquatic Diptera in many of their natural habitats (Wharton, 1959; Jenkins, 1964; Yu and Legner, 1970).

Historically, researchers have investigated planarians for their regeneration, physiology, memory and conditioning abilities, light perception, and general ecology for selected geographical areas (Mosler et al., 1967). Limited research has been done to determine effects of various chemical and physical factors on growth and reproduction of planaria. Effects of temperature on selected planaria species growth and reproduction have been studied by Legner et al.

\*The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the view of the Department of the Army or the Department of Defense.

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(1976). Other factors which have been investigated include: nutrition (Tsai and Legner, 1977), chloride concentration (Levy and Miller, 1982), photoperiod and substrate composition (Legner et al., 1976). Studies by Levy and Miller (1978) demonstrated the tolerance of the *Dugesia dorotocephala* (Woodworth) to high concentrations of pesticides and insect growth regulators. These studies led to suggestions of their possible application in an integrated pest control program.

All previous research on the physical and chemical effects on planarian growth and reproduction has been designed to study development of planarian mass production methodology for application in mosquito control. Limited quantitative data are available on the specific effects selected physical and chemical factors have on planarian growth and reproduction. The data can aid in the explanation of species diversity in various aquatic habitats (Pennak, 1978) and in the selection of species for use as biological control agents based on survival and reproductive rates in the target organism's habitat. The objective of this study was to determine effects of water quality on the survival and reproduction of *D. dorotocephala*, *D. tigrina* (Girard), *C. foremanii* (Girard), and *Dendrocelopsis vaginatus* (Hyman).

### Materials and Methods

Four species of planaria, *D. dorotocephala*, *D. tigrina*, *C. foremanii*, and *D. vaginatus*, were evaluated against six water quality parameters (hardness, temperature, salinity, pH, dissolved organics, and dissolved oxygen). All planaria were obtained from stock laboratory colonies (Carolina Biological Supply Company, Burlington, NC, USA), and species identities were confirmed using Pennak (1978). All specimens were acclimated for 48 h in artesian well water maintained in the laboratory ( $20 \pm 2^\circ\text{C}$ ) before being used in a test. Planaria used in the test were 10–15 mm in length.

Water quality parameters used in the study were based on the United States Geological Survey Service data (Hem, 1985) as representative of the various mosquito breeding sites found in the United States. All water treatments were prepared under controlled laboratory conditions from artesian well water with the exception of hardness, in which artificial medium (Marking and Dawson, 1973) was used. The water quality parameters of the well water were a hardness of 131 mg/l  $\text{CaCO}_3$ , salinity of 25 ppm  $\text{Cl}$ , pH of 7.6,

dissolved carbon of 5 mg/l, and a dissolved oxygen level of 8 ppm.

Exposure to hardness, temperature, salinity, dissolved organics, and dissolved oxygen were done under static with 48 h renewal conditions. Standard United States Environmental Protection Agency analytical methods were used for water analysis (EPA, 1986). Hardness was determined by a titration procedure as mg/l  $\text{CaCO}_3$  (Chemetrics, Inc., Calverton, VA), and artificial media were made (Marking and Dawson, 1973) for four water hardness levels of very soft (10–13 mg/l  $\text{CaCO}_3$ ), soft (40–48 mg/l  $\text{CaCO}_3$ ), hard (160–168 mg/l  $\text{CaCO}_3$ ), and very hard (280–320 mg/l  $\text{CaCO}_3$ ). Salinity was determined by ion chromatography as ppm  $\text{Cl}^-$  (Dionex 4000 Ion Chromatograph, Sunnyvale, CA). Ten salinity levels (25, 50, 100, 250, 500, 950, 1000, 2000, 3000, 4000, 5000) were prepared for evaluation by adding NaCl to the well water. Total organic carbon was determined with a carbon analyzer analysis as mg/l carbon (Beckman 915 Tocamaster, Fullerton, CA). Humic acid in sodium salt was used as the source of dissolved carbon for test solutions due to its high solubility in water and no effect on water pH levels. Dissolved oxygen was determined by a dissolved oxygen meter as parts per-million dissolved  $\text{O}_2$  (Orion Oxygen Meter, Cambridge, MA). Oxygen levels were adjusted downward from air saturated solutions by bubbling nitrogen gas into the water and were maintained within 2% of the designated levels in 50 ml glass vials with teflon-lined caps. All bioassay containers were maintained at  $20 \pm 1^\circ\text{C}$  except for those used to determine effects of temperature. These were kept in environmentally controlled rooms adjusted to maintain the various water test temperatures. Rooms were otherwise identically maintained at  $80 \pm 5\%$  RH and a 16 h photoperiod.

Evaluation of the effects of pH on planaria required the use of a continuous flow design system (Fig. 1) because planarian metabolic and mucous secretions alter the pH. The system consisted of a gravity fed, continuous flow design which used standard toxicological 500 ml flow-through beakers. The pH of the water was adjusted with concentrated phosphoric acid or 1 molar sodium hydroxide. One-gallon containers were used to supply the water continually by adjusting a flow valve placed on the delivery system consisting of plastic tubing of 4 mm internal diameter with an Eppendorf® tip at the end. Water was replenished daily to maintain a stable pH for the 14-day duration of the test.

## Results and Discussion

### Effects of hardness

No significant effects were determined on the survival or asexual reproduction of *D. dorotocephala* ( $F=0.61$ ,  $dF=29$ ,  $P=0.72$ ), *C. foremanii* ( $F=0.72$ ,  $dF=29$ ,  $P=0.64$ ), and *D. vaginatus* ( $F=1.85$ ,  $dF=29$ ,  $P=0.22$ ) upon exposure to the four levels of hardness. However, a trend between increased hardness and reproduction by *D. tigrina* was found ( $F=12.38$ ,  $dF=29$ ,  $P=0.002$ ) (Table 1). Since salt concentration (calcium, sodium, potassium, and magnesium) and the pH of the water increase with the level of hardness, the observed positive correlation between *D. tigrina* reproduction and water hardness suggests tolerance by this species to high salt concentration and high alkalinity.

### Effects of temperature

The main effects of temperature were found significant ( $F$ -test) for *D. dorotocephala* ( $F=3.86$ ,  $dF=29$ ,  $P=0.04$ ), *D. tigrina* ( $F=221.64$ ,  $dF=29$ ,  $P=0.0002$ ), *C. foremanii* ( $F=11.50$ ,  $dF=29$ ,  $P=0.002$ ), and *D. vaginatus* ( $F=5.50$ ,  $dF=29$ ,  $P=0.002$ ). The mean separations for results from the temperature exposure for the four species tested are shown in Table 2. *C. foremanii* and *D. vaginatus* showed significant mortality at a temperature of 30°C.

*D. dorotocephala* was determined to have the broadest temperature range for survival and actual asexual reproduction (Table 2). Legner et al. (1976) reported a lack of tolerance by this species to a water temperature above 26°C. This difference between results can be due to the reported fluctuation in temperature maintenance ( $\pm 6.3^\circ\text{C}$ ) by Legner et al. (1976) in which water baths were used, rather than the more constant temperature maintenance obtained in this study using environmentally controlled rooms.

### Effects of salinity

There was determined to be a significant main effect of salinity on *D. dorotocephala* ( $F=13.59$ ,  $dF=29$ ,  $P<0.0001$ ), *D. tigrina* ( $F=54.35$ ,  $dF=29$ ,  $P=0.0001$ ), *C. foremanii* ( $F=141.73$ ,  $dF=28$ ,  $P<0.0001$ ), and *D. vaginatus* ( $F=41.47$ ,  $dF=29$ ,  $P<0.0001$ ). Results of salinity evaluation (Table 3) show *C. foremanii* as the most sensitive species to salinity, tolerating a range of only 25–250 ppm  $\text{Cl}^-$  with 100% mortality at 3000 ppm.

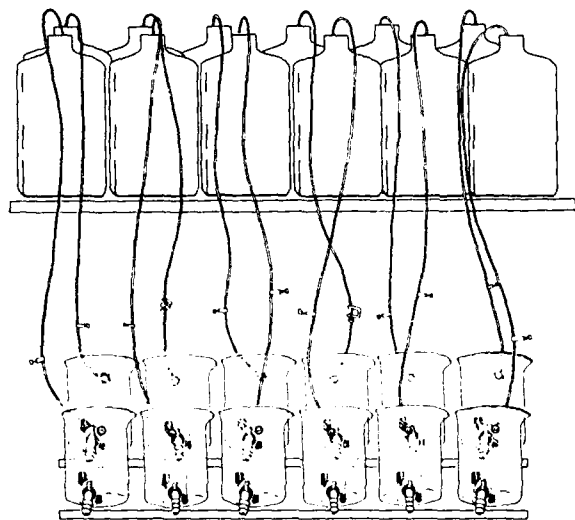


Fig. 1. Schematic of the continuous flow design system used in evaluating pH effects on the four planaria species tested.

Initially 10 planaria were randomly assigned to 100×50 mm crystallizing dishes or flow-through beakers containing 200 ml of the various water treatments. Each test was replicated three times for each range of water quality parameter evaluated for a total of 30 planaria per treatment level. *D. dorotocephala* and *D. tigrina* were fed 10 third-instar *Aedes aegypti* (Linn) larvae, and *C. foremanii* and *D. vaginatus* were fed *Artemia salina* three times weekly. The duration of the exposure was 14 days, and survival and reproduction were recorded daily. A planarian was defined as dead when it did not show any response when probed with a needle. Reproduction was defined as any new planaria resulting from asexual reproduction.

Each test for each species was comprised of a balanced one-way treatment structure. The experimental design of the study was a completely randomized design; data were analyzed by an analysis of variance procedure (ANOVA,  $F$ -test [SAS/STAT, 1985]) for testing the hypothesis that main effects of survival and/or asexual reproduction of the planaria species for the various water quality parameters are due to natural variation. For those water quality treatments found to have a significant effect on the planaria species by the  $F$ -test, means were further separated using Schaffe's multiple comparison procedure (SAS/STAT, 1985) ( $P<0.05$ ).

Table 1. Effects of various hardness levels on the asexual reproduction of *Dugesia tigrina*

Specie	Mean number ( $\pm$ SEM) of planarian individuals for the indicated hardness level ( $\pm 1$ mg/l $\text{CaCO}_3$ )*			
	Very soft water 10–13 mg/l	Soft water 40–48 mg/l	Hard water 160–180 mg/l	Very hard water 280–320 mg/l
<i>Dugesia tigrina</i>	10.67 (0.52) a	11.33 (0.71) a	14.33 (0.31) ab	15.00 (0.48) b

\*Means within a row followed by the same letter are not significantly different ( $P < 0.05$ ;  $N = 30$ ; Schaffe's multiple comparison procedure [SAS Institute, 1985]).

Table 2. Effects of various temperature levels on the survival and asexual reproduction of four planarian species

Species	Mean number ( $\pm$ SEM) of planarian individuals for the indicated temperature level ( $\pm 0.5^\circ\text{C}$ )*			
	15°C	22°C	25°C	30°C
<i>Dugesia dorotocephala</i>	16.33 (1.34) a	19.00 (0.98) a	18.67 (1.21) a	18.33 (0.89) a
<i>Dugesia tigrina</i>	11.33 (0.87) bc	14.33 (1.21) a	10.67 (0.88) c	8.33 (1.08) c
<i>Cura foremanii</i>	9.33 (1.12) a	10.33 (0.95) a	10.33 (1.01) a	6.67 (1.25) b
<i>Dendrocelopsis vaginatus</i>	9.67 (0.91) a	8.67 (1.12) ab	8.67 (1.05) ab	7.67 (0.83) b

\*Means within a row followed by the same letter are not significantly different ( $P < 0.05$ ;  $N = 30$ ; Schaffe's multiple comparison procedure [SAS Institute, 1985]).

Table 3. Effects of various levels of salinity on the survival and asexual reproduction of four planarian species

Species	Mean number ( $\pm$ SEM) of planarian individuals for the indicated salinity level ( $\pm 5$ Cl ppm)*										
	25	50	100	250	500	750	1000	2000	3000	4000	5000
<i>Dugesia dorotocephala</i>	14.33a (1.11)	12.67a (0.95)	15.00a (1.18)	14.00a (0.91)	17.33a (1.15)	16.55a (0.89)	11.67ab (0.91)	8.00bc (1.03)	7.00bc (0.99)	2.33bc (1.03)	0.00c (0.71)
<i>Dugesia tigrina</i>	11.00a (0.95)	10.67a (0.95)	11.67a (1.12)	12.67a (0.93)	12.00a (0.96)	12.33a (1.21)	13.67a (1.19)	13.00a (0.87)	12.67a (1.17)	1.67b (1.11)	0.00b (0.98)
<i>Cura foremanii</i>	10.33a (1.02)	12.67a (0.81)	11.33a (1.05)	11.00a (0.95)	10.33ab (1.00)	10.33ab (0.88)	10.00ab (1.10)	8.67b (1.16)	0.00c (1.00)	0.00c (1.00)	0.00c (1.02)
<i>Dendrocelopsis vaginatus</i>	9.57a (0.94)	8.67a (0.98)	9.67a (0.99)	9.67a (0.95)	9.00a (0.97)	**	8.33a (1.09)	8.67b (0.88)	8.00a (0.91)	0.00b (0.98)	0.00b (0.87)

\*Means within a row followed by the same letter are not significantly different ( $P < 0.05$ ;  $N = 30$ ; Schaffe's multiple comparison procedure [SAS Institute, 1985]).

\*\*Not exposed at this level.

When *D. tigrina* was evaluated at four levels of hardness (Table 1), the tolerance trend suggested a tolerance by this species to high salt concentrations. Results obtained in the salinity evaluation experiments

support this observation with *D. tigrina* actually reproducing asexually through 3000 ppm  $\text{Cl}^-$ .

*D. dorotocephala* and *D. vaginatus* also tolerated up to 3000 ppm of  $\text{Cl}^-$  without significant effects.

Table 4. Effects of various pH levels on the survival and asexual reproduction of four planarian species

Species	Mean number ( $\pm$ SEM) of planarian individuals for the indicated pH level ( $\pm 1$ pH)*						
	3.0	4.0	5.0	6.0	7.0	8.0	9.0
<i>Dugesia dorocephala</i>	0b (0.35)	9.33a (0.58)	10.33a (0.98)	11.33a (0.81)	12.67a (1.12)	10.67a (1.10)	9.33a (0.59)
<i>Dugesia tigrina</i>	0b (0.57)	10.00a (1.14)	10.33a (0.89)	10.33a (0.83)	12.00a (1.03)	13.67a (0.99)	10.33a (0.83)
<i>Cura foremanii</i>	0b (0.29)	8.67a (0.35)	9.00a (0.54)	10.33a (0.71)	10.33a (0.82)	9.67a (0.91)	9.33a (0.59)
<i>Dendrocelopsis vaginatus</i>	0b (0.91)	7.00a (1.04)	8.33a (1.01)	8.67a (0.95)	7.33a (0.97)	9.00a (1.03)	7.00a (0.89)

\*Means within a row followed by the same letter are not significantly different ( $P < 0.05$ ;  $N = 30$ ; Schaffe's multiple comparison procedure [SAS Institute, 1985]).

Table 5. Effects of various dissolved carbon levels on the survival and asexual reproduction of *Dugesia dorocephala* and *Dugesia tigrina*

Species	Mean number ( $\pm$ SEM) of planarian individuals for the indicated dissolved carbon level ( $\pm 5$ mg/l)*						
	25	50	100	250	300	400	500
<i>D. dorocephala</i>	18.50a	14.00ab	14.30ab	10.33b	9.00b	11.33b	10.67b
<i>D. tigrina</i>	11.67a	11.00a	10.00a	10.00a	9.67a	10.66a	8.67b

\*Means within a row followed by the same letter are not significantly different ( $P < 0.05$ ;  $N = 30$ ; Schaffe's multiple comparison procedure [SAS Institute, 1985]).

Table 6. Effects of various levels of dissolved oxygen on the survival and asexual reproduction of four planarian species

Species	Mean number ( $\pm$ SEM) of planarian individuals for the indicated oxygen level ( $\pm 0.5$ ppm)*				
	0 ppm	2 ppm	4 ppm	6 ppm	8 ppm
<i>D. dorocephala</i>	0.00c (0.21)	0.00c (0.25)	5.33b (0.83)	6.66b (0.74)	13.33a (0.31)
<i>D. tigrina</i>	0.00d (0.81)	0.00d (0.74)	3.67c (0.69)	7.83b (0.89)	12.67a (0.91)
<i>C. foremanii</i>	0.00d (0.91)	0.00d (0.85)	2.33c (1.01)	5.00b (0.96)	8.67a (0.99)
<i>D. vaginatus</i>	0.00c (0.82)	0.00c (0.73)	0.00c (0.93)	4.67b (1.11)	9.00a (0.98)

\*Means within a row followed by the same letter are not significantly different ( $P < 0.05$ ;  $N = 30$ ; Schaffe's multiple comparison procedure [SAS Institute, 1985]).

Legner et al. (1976) reported a much lower tolerance threshold to salinity for *D. dorocephala* with 100% mortality at a  $\text{Cl}^-$  concentration of below 25 and above 2640 ppm  $\text{Cl}^-$ . Levy and Miller (1982) reported a range of 250–1000 ppm as the optimal salinity level for this species' survival. Both Legner et al. (1976) and Levy and Miller (1982) used field collected water samples from various aquatic habitats which were only analyzed for  $\text{Cl}^-$ . Additional substances such as metal salts could have caused the reported *D. dorocephala* mortality. All water used in this study was artesian well water of known quality, with only the  $\text{Cl}^-$  concentration being varied. Results from this study indicate that *D. dorocephala* may tolerate  $\text{Cl}^-$  concentrations previously reported as fatal.

### Effects of pH

Again, the main effects of pH were determined (*F*-test) to be significant and not do to variability with *D. dorocephala* ( $F=55.38$ ,  $\text{dF}=29$ ,  $P<0.0001$ ), *D. tigrina* ( $F=51.38$ ,  $\text{dF}=29$ ,  $P<0.0001$ ), *C. foremanii* ( $F=31.75$ ,  $\text{dF}=29$ ,  $P<0.0001$ ), and *D. vaginatus* ( $F=11.62$ ,  $\text{dF}=29$ ,  $P=0.0003$ ). All four species were found to survive a 4.0–9.0 pH range, with 100% mortality immediately after exposure to a pH of 3.0 (Table 4). *D. tigrina* showed the highest reproduction at a pH of 8.0, although the mean number was not significant ( $P<0.05$ ). From the other pH levels it was close ( $P=0.07$ ).

Results obtained in this part of the study were expected, due to most natural fresh waters having pH values that fluctuate from 4.0–9.0 (Hem, 1987), and freshwater organisms having the physiological adaptations to survive such fluctuations.

### Effects of dissolved carbon

The levels of dissolved carbon evaluated in this study had no observed main effects on the survival of *C. foremanii* ( $F=2.08$ ,  $\text{dF}=29$ ,  $P=0.12$ ) and *D. vaginatus* ( $F=1.69$ ,  $\text{dF}=29$ ,  $P=0.19$ ). Both *D. dorocephala* ( $F=19.91$ ,  $\text{dF}=29$ ,  $P=0.001$ ) and *D. tigrina* ( $F=3.76$ ,  $\text{dF}=29$ ,  $P=0.02$ ) were determined to be significantly effected by dissolved organics (Table 5). *D. dorocephala* showed a significant decline in reproduction at a level of 250–500 mg/l. One possible explanation for this decrease in reproduction could be insufficient nutrition due to this species inability to capture prey at such levels of dissolved organics. Legner et al. (1976) and

McConnell (1967) described the use of a mucous substance by this species to capture and kill its prey. When this substance becomes bound to the carbons in the water, it loses its adhesive proper of ties, therefore impairing the ability of *D. dorocephala* to capture and kill prey. Observations made in this study found that prey, when placed with *D. dorocephala* in lower dissolved organic water (25–100 mg/l), were consumed within 1 h after introduction. Prey placed in higher dissolved organic water (250–500 mg/l) were not affected by the planaria even 12 h after introduction.

### Effects of dissolved oxygen

The main effects of dissolved oxygen were determined (*F*-test) significant at  $P<0.0001$  for *D. dorocephala* ( $F=212.02$ ), *D. tigrina* ( $F=74.56$ ), *C. foremanii* ( $F=32.36$ ), and *D. vaginatus* ( $F=32.12$ ). Dissolved oxygen was determined to be the most critical parameter affecting both survival and reproduction of all species evaluated (Table 6) with significant mortality at 6 ppm and 100% mortality at 2 ppm.

Results from this study agree with observations by Pennak (1978) who reported that planarians in the wild will only be found in areas with oxygen levels close to saturation.

In general, the results obtained in this study confirmed the previous reports of planarian adaptability. All four species were capable of surviving both a wide range and variety of changes in water quality. *D. dorocephala* and *D. tigrina* were determined to have the highest rate of asexual reproduction at the widest ranges of temperature, salinity, and pH levels. This evidence then indicates that these two species are the best candidates for a biological control program against a variety of larval mosquito species. None of the planarian species were found to survive low dissolved oxygen levels, making none viable biological control agents against mosquito species with larval habitats having low oxygen levels such as sewage lagoons.

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